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Effect of Inoculum Size on Detection of *Candida* Growth by the BACTEC 9240 Automated Blood Culture System Using Aerobic and Anaerobic Media

Benjamin J. George, Lynn L. Horvath,* and Duane R. Hospenthal

Department of Medicine, Brooke Army Medical Center, Fort Sam Houston, Texas

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Simulated candidemia was produced with 20 *Candida* isolates at three inoculum sizes (100, 10, and 1 CFU/ml of blood). Growth detection was better with larger inocula. The time to growth detection was shorter with larger inocula. Inoculum size does effect *Candida* growth detection and time to detection in BACTEC 9240 automated systems.

Candida species rank fourth among organisms isolated from the blood of hospitalized patients, with non-*albicans Candida* species accounting for about 50% of these *Candida* isolates (1, 2, 5, 9, 11, 14). Most hospitals rely on automated blood culture systems for the detection of blood pathogens, including *Candida* spp. The College of American Pathologists recommends that blood cultures be incubated for 5 days and then discarded without terminal subculture if growth is not detected by the automated system. In two previous studies of simulated candidemia with the BACTEC 9240 automated blood culture system (Becton Dickinson and Co., Sparks, Md)., we noted poor detection in anaerobic medium, false-negative results on terminal subculture, and prolonged times to growth detection for *C. glabrata* in aerobic medium despite the use of a large inoculum (100 CFU/ml of blood) (7, 8).

It has been suggested that patients with candidemia have <10 CFU/ml of blood. Simulation of candidemia is not standardized throughout the literature, with inoculum sizes ranging from <1 to >100 CFU/ml of blood (6, 7, 8, 16, 18). Furthermore, the inoculum recommended by the manufacturer for quality control testing of the BACTEC 9240 system, and similar automated systems, is exponentially larger than what would be expected to exist in clinical candidemia (approximately 1,000 organisms/bottle). This study was therefore conducted to determine if inoculum size affected *Candida* growth detection or time to detection in the BACTEC 9240 system.

(This work was presented in part previously [B. J. George, L. L. Horvath, and D. R. Hospenthal, 103rd Gen. Meet. Am. Soc. Microbiol., abstr. C-267, 2003].)

Twenty *Candida* isolates, including four each of *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*, were evaluated. Yeast suspensions of 10^6 CFU/ml were produced as described in NCCLS antifungal susceptibility testing guidelines and our previous publications (7, 8, 12). Serial dilutions of the suspensions were performed, and dilution accuracy was confirmed as previously described (7, 8).

Sixty aerobic (BACTEC Plus Aerobic/F) and 60 anaerobic (BACTEC Plus Anaerobic/F) blood culture bottles (Becton Dickinson and Co.) were inoculated with 10 ml of fresh, unprocessed blood from healthy volunteers and then with a 0.1-ml aliquot of yeast suspension, resulting in final inoculum sizes of approximately 1, 10, and 100 CFU/ml of blood and 10, 100, and 1000 CFU/bottle, respectively. Prepared bottles were placed in the BACTEC 9240 system and incubated at 35°C.

Once growth was detected by the BACTEC 9240 system, each bottle was removed from the system and a terminal subculture was performed as previously described (7, 8). Terminal subculture was performed for all bottles without growth detection after 17 days of incubation. Data analysis was performed using SPSS software version 11.5 (SPSS Inc., Chicago, Ill.).

The BACTEC 9240 system detected *Candida* growth in 68 of 120 bottles (57%) (Table 1). Growth detection was better in aerobic medium (59 of 60 bottles [98%]) than in anaerobic medium (9 of 60 bottles [15%]) ($P < 0.001$). The BACTEC 9240 system detected all *C. albicans*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* isolates in aerobic medium, regardless of inoculum size. *C. glabrata* and *C. tropicalis* were the only species with growth detected in anaerobic medium. Overall, there was a trend towards better *Candida* growth detection with increasing inoculum size that did not reach statistical significance: 25

TABLE 1. Number of *Candida* isolates with growth detected by the BACTEC 9240 system

Species (no. of isolates)	No. of isolates detected in:					
	Plus Aerobic/F medium[a]			Plus Anaerobic/F medium		
	10[b]	100	1,000	10	100	1,000
C. albicans (4)	4	4	4	0	0	0
C. glabrata (4)	3	4	4	2	2	3
C. krusei (4)	4	4	4	0	0	0
C. parapsilosis (4)	4	4	4	0	0	0
C. tropicalis (4)	4	4	4	0	0	2
Total (20)[c]	19	20	20	2	2	5

[a] Overall detection in aerobic medium (98%) was significantly better than that in anaerobic medium (15%) ($P < 0.001$).
[b] Inoculum size in CFU per blood culture bottle.
[c] Of 120 bottles, growth was detected in 68 (57%) by the BACTEC 9240 system.

* Corresponding author. Mailing address: Brooke Army Medical Center, Infectious Disease Service (MCHE-MDI), 3851 Roger Brooke Dr., Fort Sam Houston, TX 78234. Phone: (210) 916-4355. Fax: (210) 916-0388. E-mail: Lynn.Horvath@amedd.army.mil.

TABLE 2. Mean time to *Candida* growth detection by the BACTEC 9240 system

Species	Mean time to growth detection ± SD (h) in:					
	Plus Aerobic/F medium			Plus Anaerobic/F medium		
	10[a]	100	1,000	10	100	1000
C. albicans	35.89 ± 8.32	29.67 ± 1.16	21.00 ± 3.47	NGD[b]	NGD	NGD
C. glabrata[c]	155.69 ± 57.17	128.17 ± 32.23	99.33 ± 30.37	42.03 ± 10.59	38.85 ± 11.38	25.70 ± 3.33
C. krusei	23.73 ± 1.88	21.32 ± 1.70	18.23 ± 2.24	NGD	NGD	NGD
C. parapsilosis	32.06 ± 1.70	28.27 ± 1.85	23.85 ± 0.46	NGD	NGD	NGD
C. tropicalis	24.09 ± 4.50	19.57 ± 2.20	15.75 ± 1.62	NGD	NGD	19.27 ± 2.40

[a] Inoculum size in CFU per blood culture bottle.
[b] NGD, no growth detected.
[c] For *C. glabrata*, the time to detection was significantly longer in aerobic medium than in anaerobic medium ($P < 0.001$) and longer than for all *Candida* spp. in aerobic medium ($P < 0.05$).

of 40 (63%) bottles with growth detected at 1,000 CFU/bottle, 22 of 40 (55%) bottles at 100 CFU/bottle, and 21 of 40 (53%) bottles at 10 CFU/bottle.

One hundred percent of the 68 bottles with growth detected in the BACTEC 9240 system grew yeast cells on terminal subculture, representing true-positive results. Of the 52 bottles without growth detection by the BACTEC 9240 system, 42 (81%) grew yeast cells on terminal subculture. Terminal subculture detected more *Candida* growth (110 of 120 bottles or 92%) than the BACTEC 9240 system did (68 of 120 bottles or 57%) ($P < 0.05$). Overall, 42 of 120 bottles had yeast growth upon terminal subculture that was not detected by the BACTEC 9240 system (35% false negatives). Ten bottles had no growth detected by either the BACTEC 9240 system or terminal subculture. All 10 of these bottles had been inoculated with the smallest yeast inoculum (10 CFU/bottle). Colony counts confirmed the inoculum size, with a mean of 9.6 CFU/bottle for all *Candida* isolates. Eight of the 10 bottles with no growth by either method contained less than the average 9.6 CFU/bottle. However, each of these 10 isolates grew in their corresponding bottles with the same small inoculum.

Time to growth detection by the BACTEC 9240 system varied depending on inoculum size, species, and medium (Table 2). Time to growth detection was shorter with larger inoculum ($P < 0.001$). For *C. glabrata* isolates, growth was detected earlier in anaerobic than aerobic medium with similar inoculum sizes ($P < 0.001$). The mean time to growth detection of *C. glabrata* in aerobic medium was also longer than that for any other *Candida* species at similar inoculum sizes ($P < 0.05$).

Candida spp. are generally regarded as obligate aerobes (19); therefore, it is not surprising that the majority of bottles with undetected growth contained anaerobic medium. However, it is troubling that *C. glabrata* growth went undetected even in a single aerobic bottle. Clinical laboratories must be concerned about time to growth detection, as most limit the duration of blood culture incubation to 5 days due to space and monetary constraints. There are data indicating that 3 days may be sufficient for recovery of 97.5% of clinically significant pathogens in the BacT/ALERT automated blood culture system, including *Candida* spp. (4). Most of the *Candida* spp. evaluated in this study were detected within either a 3- or 5-day incubation period regardless of inoculum size, except for *C. glabrata*, which required incubation for longer than 5 days at the two smallest inoculum sizes in aerobic medium.

There are limited data on the usefulness of terminal subculture for detection of candidemia in conjunction with automated systems (3, 13, 17). It is notable that 81% of the bottles with *Candida* spp. not detected by the BACTEC 9240 system had yeast growth upon terminal subculture. Missed detection of growth is particularly concerning, since all of these bottles contained inocula of sizes similar to the levels encountered in human candidemia (16). While inoculum size did affect growth detection and time to growth detection, inoculum size alone does not explain the inability of this system to detect growth of viable yeasts.

The BACTEC 9240 system was able to detect the majority of simulated candidemia episodes at all inoculum sizes. However, this study illustrates several potential problems, including difficulty detecting *Candida* growth in anaerobic medium at all inoculum sizes, longer time to detection with small inoculum sizes, and the unusual growth characteristics of *C. glabrata.* This is a particular problem since *C. glabrata* is the second most common *Candida* species isolated in U.S. hospitals, accounting for 24 to 42% of all cases of candidemia (5, 14). These problems raise the question of whether clinical laboratories should prolong the incubation time, perform terminal subcultures, or utilize specific mycology media for the detection of *Candida* when utilizing the BACTEC 9240 system (3, 7, 8, 10, 13, 15, 17). With an increasing incidence of candidemia from non-*albicans Candida* spp., automated systems need to detect all clinically relevant candidal pathogens or laboratories will have to consider adjusting their protocols accordingly.

REFERENCES

1. **Beck-Sagué, C. M., W. R. Jarvis, and the National Nosocomial Infections Surveillance System.** 1993. Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980–1990. J. Infect. Dis. **167:**1247–1251.
2. **Berenguer, J., M. Buck, F. Witebsky, F. Stock, P. A. Pizzo, and T. J. Walsh.** 1993. Lysis-centrifugation blood cultures in the detection of tissue-proven invasive candidiasis. Disseminated versus single-organ infection. Diagn. Microbiol. Infect. Dis. **17:**103–109.
3. **Borst, A., M. Leverstein-Van Hall, J. Verhoef, and A. Fluit.** 2000. Value of terminal subculture of automated blood cultures in patients with candidaemia. Eur. J. Clin. Microbiol. Infect. Dis. **19:**803–805.
4. **Bourbeau, P. P., and J. K. Pohlman.** 2001. Three days of incubation may be sufficient for routine blood cultures with BactT/Alert FAN blood culture bottles. J. Clin. Microbiol. **39:**2079–2082.
5. **Edmond, M. B., S. E. Wallace, D. K. McClish, M. A. Pfaller, R. N. Jones, and R. P. Wenzel.** 1999. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. Clin. Infect. Dis. **29:**239–244.

6. **Fricker-Hidalgo, H., F. Chazot, B. Lebeau, H. Pelloux, P. Ambroise-Thomas, and R. Grillot.** 1998. Use of simulated blood cultures to compare a specific fungal medium with a standard microorganism medium for yeast detection. Eur. J. Clin. Microbiol. Infect. Dis. **17:**113–116.
7. **Horvath, L. L., D. R. Hospenthal, C. K. Murray, and D. P. Dooley.** 2003. Detection of simulated candidemia by the BACTEC 9240 system with Plus Aerobic/F and Anaerobic/F blood culture bottles. J. Clin. Microbiol. **41:** 4714–4717.
8. **Horvath, L. L., B. J. George, C. K. Murray, L. S. Harrison, and D. R. Hospenthal.** 2004. Direct comparison of BACTEC 9240 and BacT/ALERT 3D automated blood culture systems for *Candida* growth detection. J. Clin. Microbiol. **42:**115–118.
9. **Maksymiuk, A. W., S. Thongprasert, R. Hopfer, M. Luna, V. Fainstein, and G. P. Bodey.** 1984. Systemic candidiasis in cancer patients. Am. J. Med. **77:**20–27.
10. **Meyer, M. H., V. Letscher-Bru, B. Jaulhac, J. Waller, and E. Candolfi.** 2004. Comparison of Mycosis IC/F and Plus Aerobic/F media for diagnosis of fungemia by the Bactec 9240 system. J. Clin. Microbiol. **42:**773–777.
11. **Myerowitz, R. L., G. J. Pazin, and C. M. Allen.** 1977. Disseminated candidiasis. Changes in incidence, underlying disease, and pathology. Am. J. Clin. Pathol. **68:**29–38.
12. **NCCLS.** 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard, 2nd ed. NCCLS document M27-A2. NCCLS, Wayne, Pa.
13. **Prevost-Smith, E., and N. Hutton.** 1992. Value of extended agitation and subculture of BACTEC NR 660 aerobic resin blood culture bottles for clinical yeast isolates. J. Clin. Microbiol. **30:**3239–3242.
14. **Rangel-Frausto, M. S., T. Wiblin, H. M. Blumberg, L. Saiman, J. Patterson, M. Rinaldi, M. Pfaller, J. E. Edwards, Jr., W. Jarvis, J. Dawson, R. P. Wenzel, and the NEMIS Study Group.** 1999. National epidemiology of mycoses survey (NEMIS): variations in rates of bloodstream infections due to *Candida* species in seven surgical intensive care units and six neonatal intensive care units. Clin. Infect. Dis. **29:**253–258.
15. **Reisner, B. S., and G. L. Woods.** 1999. Times to detection of bacteria and yeasts in BACTEC 9240 blood culture bottles. J. Clin. Microbiol. **37:**2024–2026.
16. **Schelonka, R. L., M. K. Chai, B. A. Yoder, D. Hensley, R. M. Brockett, and D. P. Ascher.** 1996. Volume of blood required to detect common neonatal pathogens. J. Pediatr. **129:**275–278.
17. **Shigei, J. T., J. A. Shimabukuro, M. T. Pezzlo, L. M. de la Maza, and E. M. Peterson.** 1995. Value of terminal subcultures for blood cultures monitored by BACTEC9240. J. Clin. Microbiol. **33:**1385–1388.
18. **Vigano, E. F., E. Vasconi, C. Agrappi, and P. Clerici.** 2002. Use of simulated blood cultures for time to detection comparison between BacT/ALERT and BACTEC 9240 blood cultures systems. Diagn. Microbiol. Infect. Dis. **44:** 235–240.
19. **Weinstein, M. P., S. Mirrett, L. B. Reller, L. G. Reimer, and M. L. Wilson.** 1996. Value of terminal subcultures for blood cultures monitored by BACTEC 9240. J. Clin. Microbiol. **34:**234–235.